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Data Sheet
IDO1 Fluorogenic Inhibitor Screening Assay Kit
(384 Well Format)
Catalog #72047

DESCRIPTION: The *IDO1 Fluorogenic Inhibitor Screening Assay Kit* is designed to measure enzyme inhibition of indoleamine 2,3-dioxygenase 1 (IDO1). The kit comes in a convenient format, with enough reaction solution and enzyme to perform a total of 400 reactions. The *IDO1 Fluorogenic Inhibitor Screening Assay Kit* is simple to use and detects fluorescence at long wavelengths, which minimizes potential errors due to compound interference. In the assay, the inhibitor and enzyme are added to a sample containing L-Trp substrate. After a 1 hour incubation at room temperature, the fluorescence solution is added and incubated at 37°C for four hours. Activity is measured by reading sample fluorescence at $\lambda=510$ nm following excitation of the reaction product at $\lambda=400$ nm.

BACKGROUND: L-tryptophan (L-Trp) is an essential amino acid necessary for protein synthesis in mammalian cells and the L-Trp to kynurenine (Kyn) pathway is firmly established as a key regulator of innate and adaptive immunity. Catabolism of L-Trp to Kyn maintains an immunosuppressive microenvironment by starving immune cells of L-Trp and releasing degradation products of L-Trp that have immunosuppressive functions. Indoleamine 2,3-dioxygenases (IDO1 & IDO2), two of the rate limiting enzymes in this pathway, are upregulated in many tumors, providing cancer cells with an avenue for immune evasion.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71182	IDO1 His-Tag	2 x 80 μ g	-80°C	(Avoid freeze/thaw cycles!)
73009	IDO1 Fluorogenic Reaction Solution	5 x 10 ml	-80°C	
73002	IDO1 Assay Buffer	5 ml	-80°C	
	Fluorescence Solution	7 x 1 ml	-80°C	
	Black 384 Well Assay-Plate	1		
	Plate sealing film	1		

MATERIALS REQUIRED BUT NOT SUPPLIED:

Fluorimeter capable of excitation at 390-410 nm and detection at 500-520 nm
 Adjustable micropipettor and sterile tips
 Rotating or rocker platform

APPLICATIONS: Useful for the study of IDO1 enzymology, inhibitor screening, and selectivity profiling.

STABILITY: At least one year from date of receipt when stored as directed.

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CONTRAINDICATIONS: DMSO >0.5%, strong acids or bases, ionic detergents, high salt.
Warning: the Fluorescence Solution contains a component that is known to be a skin and eye irritant. Use caution and appropriate personal protective equipment when handling this component.

REFERENCE(S):

1. Seegers, N., et al. *J. Biomol. Screen.* 2014. **19(9)**:1266-74.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate. Use slow shaking for all incubations.

Step 1:

- 1) Thaw reaction solution and aliquot 90 μ l into each well.
- 2) Add 5 μ l of inhibitor solution (no more than 10% DMSO) to each well designated "Test Inhibitor." For the wells designated "Positive Control" and "Blank," add 5 μ l of the same solution without inhibitor (inhibitor buffer). Note: Keep the final DMSO concentration below 0.5%.
- 3) Dilute **IDO1 His-Tag** in **IDO1 Assay Buffer** at 80 ng/ μ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Positive Control	Test Inhibitor
IDO1 Fluorogenic Reaction Solution	90 μ l	90 μ l	90 μ l
Test Inhibitor	-	-	5 μ l
Inhibitor buffer (no inhibitor)	5 μ l	5 μ l	-
IDO1 Assay Buffer	5 μ l	-	-
IDO1 His-Tag (80 ng/ μ l)	-	5 μ l	5 μ l
Total	100 μl	100 μl	100 μl

- 4) Add 5 μ l of **IDO Assay Buffer** to the wells designated "Blank."
- 5) Initiate reaction by adding 5 μ l of diluted **IDO1 His-Tag** prepared as described above to the wells labeled "Positive Control," and "Test Inhibitor." Incubate at room temperature for 1 hour.
- 6) Add 16 μ l **Fluorescence Solution** to each well. Seal the plate and incubate at 37°C for four hours. Allow plate to cool for at least 10 minutes.
- 7) Unseal the plate. For best results, centrifuge plate to reduce condensation on the film. Inspect plate for wells with large changes in volume. Wells with significant changes

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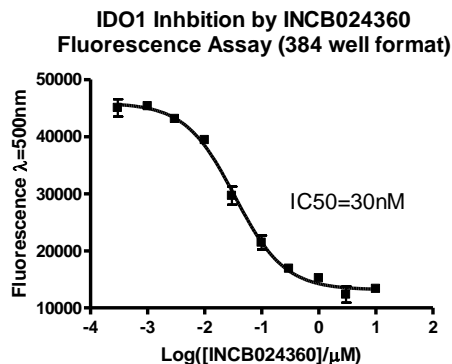
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should be excluded from data analysis. Measure fluorescence in a fluorimeter capable of excitation at 400 nm and emission at 510 nm. Subtract "Blank" value from all other values.

EXAMPLE OF ASSAY RESULTS:



Inhibition of IDO1 activity by INCB024360 (Cat. #27338), measured using the *IDO1 Fluorogenic Inhibitor Screening Assay Kit (384 well format)*, BPS Bioscience, Catalog #72047. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

RELATED PRODUCTS:

<u>Product</u>	<u>Catalog #</u>	<u>Size</u>
IDO1, His-tag	71182	50 μg
IDO2, His-tag	71194	50 μg
TDO, His-tag	71195	50 μg
IDO1 Inhibitor Screening Assay Kit	72021	96 rxns
IDO2 Inhibitor Screening Assay Kit	72022	96 rxns
TDO Inhibitor Screening Assay Kit	72023	96 rxns
IDO1 Cell-Based Assay Kit	72031	100 rxns
TDO Cell-Based Assay Kit	72033	100 rxns
IDO1-HEK293 Recombinant Cell line	60532	2 vials
IDO1 Cellular Activity QuickDetect™ Supplements	62000-1	100 rxns
N-formylkynurenine	73000	2 mg
NLG919	27337-1	10 mg
INCB024360	27338-1	10 mg
IDO1 Reaction Solution	73001	10 ml
IDO1 Assay Buffer	73002	1 ml
IDO2 Reaction Solution	73003	10 ml
IDO2 Assay Buffer	73004	1 ml

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